TABLE 1. Sequences used during SELEX.

(all are shown in a 5' to 3' direction, and separated by a blank every 10 bases)

Sequences involved in SELEX process:

(P0; DNA template for round 0 of spot SELEX)

TCGGGCGAGT CGTCTGNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN 50

NNNNNNCCGC ATCGTCCTCC C 71 (SEQ ID NO: 1)

A=dA; C=dC; G=dG; T=dT; N+25% each of dA, dC, dG, or dT

(5'N7; primer used in PCR steps of SELEX)

TAATACGACT CACTATAGGG AGGACGATGC GG 32 (SEQ ID NO: 2)

A=dA; C=dC; G=dG; T=dT

(3'N7; primer used in RT and PCR steps of SELEX)

TCGGGCGAGT CGTCTG 16 (SEQ ID NO: 3)

A=dA; C=dC; G=dG; T=dT

(Transcription template for round 0 of spot SELEX)

TAATACGACTCACTATAGGGAGGACGATGCGG-40N-CAGACGACTCGCCCGA 88 bp (SEQ ID NO:4)

ATTATGCTGAGTGATATCCCTCCTGCTACGCC-40N-GTCTGCTGAGCGGGCT (SEQ ID NO: 5)

A=dA; C=dC; G=dG; T=dT; N=25% each of dA, dC, dG, or dT

(R0 40N7; nucleic acid library for round 0 of spot SELEX)

GGGAGGACGA UGCGGNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN 50

NNNNNCAGAC GACUCGCCCG A 71 (SEQ ID NO: 6)

A=2'-OH A; C=2'-F C; G=2'-OH G; N=25 % each of 2'-OH A, 2'-F C, 2'-OH G, and 2'-F U; U=2'-F U

TABLE 1 CONT. Sequences used during SELEX.

(34N7.21a-21 DNA template for round 0 of biased SELEX)

GGGAGGACGA TGCGGNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNC 50

AGACGACTCG CCCGA 65 (SEQ ID NO: 7)

A=dA; C=dC; G=dG; T=dT, N=62.5 % NX22284 sequence as DNA and 12.5% of the other 4 nucleotides (dA, dC, dG, or dT) at each position

(Transcription template for round 0 of biased SELEX)

TAATACGACTCACTATAGGGAGGACGATGCGG-34N-CAGACGACTCGCCCGA 82 bp (SEQ ID NO: 8)

ATTATGCTGAGTGATATCCCTCCTGCTACGCC-34N-GTCTGCTGAGCGGGCT (SEQ ID NO: 9)

A=dA; C=dC; G=dG; T=dT, N=62.5 % NX22284 sequence as DNA and 12.5% of the other 4 nucleotides (dA, dC, dG, or dT) at each position

(34N7.21a-21 nucleic acid library for round 0, biased SELEX)

GGGAGGACGA UGCGGNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNC 50

AGACGACUCG CCCGA 65 (SEQ ID NO: 10)

A=2'-OH A; C=2'-F C; G=2'-OH G; N=62.5 % NX22284 sequence and 12.5% of other 4 nucleotides (2'-OH A, 2'-F C, 2'-OH G, or 2'-F U) at each position; U=2'-F U

<u>Sequences used for subcloning, screening, sequencing ligand</u>

(ML-34; used for subcloning)

CGCAGGATCC TAATACGACT CACTATA 27 (SEQ ID NO: 11)

A=dA; C=dC; G=dG; T=dT

(ML-78; used for subcloning)

TABLE 1 CONT. Sequences used during SELEX.

GGCAGAATTC TCATCTACTT AGTCGGGCGA GTCGTCTG (SEQ ID NO: 12)
A=dA; C=dC; G=dG; T=dT

(RSP1 ; vector-specific primer used to screen transformants for ligand inserts)
AGCGGATAAC AATTTCACAC AGG 23 (SEQ ID NO: 13)
A=dA; C=dC; G=dG; T=dT

(FSP2; vector-specific primer used to screen transformants for ligand inserts)
GTGCTGCAAG GCGATTAAGT TGG 23 (SEQ ID NO: 14)
A=dA; C=dC; G=dG; T=dT

(RSP2; primer for sequencing ligands)
ACTTTATGCT TCCGGCTCG 19 (SEQ ID NO: 15)
A=dA; C=dC; G=dG; T=dT

Sequences used to detect specific ligands

(ligand 14i-1 specific primer; ML85)
GCCAAATGCC GAGAGAACG 19 (SEQ ID NO: 16)
A=dA; C=dC; G=dG; T=dT

(ligand 21a-4 specific primer; ML-79)
GGGGACAAGC GGACTTAG 18 (SEQ ID NO: 17)
A=dA; C=dC; G=dG; T=dT

(ligand 21a-21 specific primer; ML-81)
GGGAGTACAG CTATACAG 18 (SEQ ID NO: 18)
A=dA; C=dC; G=dG; T=dT

TABLE 1 CONT. Sequences used during SELEX.

Sequences used for RNAse H cleavage

(5'N7 cleave)

CCGCaugcuc cuccc 15 (SEQ ID NO: 19)

a=2'-OCH_3 A; c=2'-OCH_3 C; C=dC; g=2'-OCH_3 G; G=dG; u=2'-OCH_3 U

(3'N7 cleave)

ucgggcgagu cgTCTG 16 (SEQ ID NO: 20)

a=2'-OCH_3 A; c=2'-OCH_3 C; C=dC; g=2'-OCH_3 G; G=dG; u=2'-OCH_3 U; T=dT

TABLE 2. Conditions and results of filter SELEX

Round[a]	[RNA][b], nM	[TGFβ2], nM	RNA[b]/protein	[Competitor]	% Bound	% Background	Bound/Background	Kd (nM)
9b	1 nM	100 nM	0.01	100 μM tRNA	4.2	1.1	4	nd
10b	1 nM	30 nM	0.03	100 μM tRNA	4.3	0.13	33	100
11a	1 nM	30 nM	0.03	100 μM tRNA	1.5	0.2	8	75
12d	0.2 nM	20 nM	0.01	250 μM tRNA	2.2	0.3	7	40
13i	0.4 nM	10 nM	0.04	10 μM tRNA	2.6	0.16	16	30
14i	0.1 nM	10 nM	0.01	10 μM heparin	14.5	0.55	20	75
15c	10 nM	10 nM	1.0	0	8.8	2.2	4	30
16a	55 nM	10 nM	5.5	0	9.6	2.1	5	10
17a	30 nM	3 nM	10	0	1.9	0.17	11	5
18b	15 nM	3 nM	5	0	2.3	0.6	4	5
19a	7 nM	0.1 nM	70	0	0.17	0.05	3	2
20a	0.33 nM	0.03 nM	11	0	0.1	0.04	3	1
21a	0.63 nM	0.03 nM	21	0	0.3	0.1	3	1
22a	0.07 nM	0.01 nM	7	0	0.12	0.09	1	1

[a]Number designates the round of SELEX and letter designates the condition used for that round.

[b]NA, nucleic acid library

Only those rounds that were carried to the next round are shown

TABLE 3. Conditions and results of Spot SELEX

Rd	Protein (pmoles)	RNA (pmoles)	Washes[1] (µl/min)	Signal/ Noise	% Input	Incubation	Pre-adsorb[2]
1	*200	2000	2 (500/10)	4.90	ND[3]	4 hrs, 20°C	No
2	*200	1500	2 (1000/10)	1.80	ND	0.5 hrs, 37°C	5 layers, 0.75hrs
3	*200	1500	2 (1000/10)	5.50	ND	1 hr, 37°C	5 layers, 1 hr
4	200	1000	2 (1000/10)	11.20	0.18	1 hr, 37°C	5 layers, 2.5 hrs
	*67	1000	2 (1000/10)	3.70	0.06	1 hr, 37°C	5 layers, 2.5 hrs
	22	1000	2 (1000/10)	1.58	0.03	1 hr, 37°C	5 layers, 2.5 hrs
5	67	100	2 (1000/20)	26.00	1.30	1 hr, 37°C	10 layers, 0.75hrs
	*22	100	2 (1000/20)	11.00	0.56	1 hr, 37°C	10 layers, 0.75hrs
	7.3	100	2 (1000/20)	2.70	0.10	1 hr, 37°C	10 layers, 0.75hrs
6	22	50	2 (1000/20)	20.70	1.00	1 hr, 37°C	10 layers, 0.75hrs
	*7.3	50	2 (1000/20)	4.00	0.20	1 hr, 37°C	10 layers, 0.75hrs
	2.4	50	2 (1000/20)	1.20	0.06	1 hr, 37°C	10 layers, 0.75hrs
7	22	7	3 (1000/50)	24.00	1.30	1 hr, 37°C	10 layers, 1.5hrs
	*7.3	7	3 (1000/50)	7.50	0.40	1 hr, 37°C	10 layers, 1.5hrs
	2.4	7	3 (1000/50)	1.50	0.07	1 hr, 37°C	10 layers, 1.5hrs
8	*7.3	3	2 (1000/60)	77.00	0.41	0.75 hr, 37°C	10 layers, 1.5hrs
	2.4	3	2 (1000/60)	8.50	0.04	0.75 hr, 37°C	10 layers, 1.5hrs
	0.7	3	2 (1000/60)	1.00	ND	0.75 hr, 37°C	10 layers, 1.5hrs
9	*7.3	1	2 (1000/20)	87.00	0.23	1 hr, 37°C	10 layers, 1.5hrs
	2.4	1	2 (1000/20)	4.00	0.01	1 hr, 37°C	10 layers, 1.5hrs
	0.7	1	2 (1000/20)	2.50	0.006	1 hr, 37°C	10 layers, 1.5hrs
10	7.3	<1 (no tRNA)	2 (1000/20)	13.70	ND	0.5 hr, 37°C	10 layers, 1.5hrs
	7.3	<1 (10^1 tRNA)[4]	2 (1000/20)	10.50	ND	0.5 hr, 37°C	10 layers, 1.5hrs
	7.3	<1 (10^2 tRNA)	2 (1000/20)	5.00	ND	0.5 hr, 37°C	10 layers, 1.5hrs
	7.3	<1 (10^3 tRNA)	2 (1000/20)	1.80	ND	0.5 hr, 37°C	10 layers, 1.5hrs

*pool carried to next round

[1]Number of washes, volumes and duration

[2]Number of filters and duration of incubation during the background counterselection step

[3]ND, not determined

[4]Fold excess tRNA over the aptamer pool

TABLE 4. Conditions and results surface plasmon resonance biosensor (spr) SELEX.

Progress of BIA SELEX with TGFβ2

Rd	**TGFβ2, RU[1]**				**[RNA], μM[2]**	**Injections (vol, μL)[3]**	**Fractions (min each)[4]**	**Fraction FW[5]**	**RU after SDS[6]**
	FC1	FC2	FC3	FC4					
2	1293	874	294	0	4	4 (40)	3 (5)	3rd & SDS	~100
3	1176	1178	1181	0	15	4 (40)	3 (5)	3rd & SDS	~50-100
4	3010	2037	1767	0	10	6 (40)	3 (5)	3rd & SDS	~80
5	5520	5334	4265	0	5	6 (40)	3 (5)	3rd & SDS	~100-150
6	4075	3143	298	0	5	6 (40)	3 (5)	3rd & SDS	~75-100
7	3773	2616	2364	0	2	6 (40)	3 (5)	3rd & SDS	~330-220
8	2574	1842	1461	0	5	4 (40)	3 (5)	3rd & SDS	~60-105
9	3180	2029	1688	0	3	4 (40)	3 (5)	3rd & SDS	~77-114
10	344	718	1692	0	1	4 (40)	6 (10)	6th & SDS	~50
11	217	675	386	0	5	2 (40)	6 (10)	6th & SDS	~50-62

[1]Amount of TGFβ2 immobilized expressed in resonance units where 1RU corresponds to 1pg of protein per mm^2. The protein is immobilized in an area of 1.2 mm^2

[2]concentration of RNA pools

[3]Number of injections and volume of each injection

[4]Number and length in min (in parentheses) of each fraction

[5]Fractions carried to the next round

[6]Amount of RNA eluted after SDS treatment expressed in response units

FC1, FC2, FC3, and FC4 designate the four flowcells of the BIA chip.

TABLE 5. Sequences isolated from round 8 of surface plasmon resonance SELEX.

NAME[a]	SEQ ID NO.	SEQUENCE[b]	BINDING[c]
8.1(1)	21	GGGAGGACGAUGCGG UCCUCAAUG-AUCUU---------UCCUGUUUAUGCUCCC CAGACGACUCGCCCGA	FILTER
8.2(1)	22	GGGAGGACGAUGCGG AAGUAACGUUUA AGUAAAAUUCGUUCUCUCGGU AUUUGGC CAGACGACUCGCCCGA	TGFβ2
8.3(14)	23	GGGAGGACGAUGCGG AAGUAACGUUGA AGUAAAAUUCGUUCUCUCGGC AUUUGGC CAGACGACUCGCCCGA	TGFβ2
8.5(1)	24	GGGAGGACGAUGCGG UCCUAACCAUCACAAUCUCAAUUCUUAUAUUUUCCCGCCC CAGACGACUCGCCCGA	NONE
8.6(1)	25	GGGAGGACGAUGCGG --AAACCAAAAGACCACAUCUCCAUACUCACGCUCUGCCC CAGACGACUCGCCCGA	NONE
8.8(1)	26	GGGAGGACGAUGCGG AUAGAUCGGUCCGAUAAGUCUUUCAUCUUUACCUGGCCCC CAGACGACUCGCCCGA	NONE
8.9(4)	27	GGGAGGACGAUGCGG AAGUAACGUUGA AGUAAAAUUCGUUCUCUCGGU AUUUGGC CAGACGACUCGCCCGA	TGFβ2
8.11(1)	28	GGGAGGACGAUGCGG ACGAUCCUUUCCUUAACAUUUCAUCAUUUCUCCUGUGCCC CAGACGACUCGCCCGG	FILTER
8.12(1)	29	GGGAGGACGAUGCGG UCCAUCAACAAUCUUAUCAUUAUGUUUUUCCUUCCCGCCC CAGACGACUCGCCCGA	NONE
8.13(1)	30	GGGAGGACGAUGCGG UCCUCUGAGCCGAUCUUCUUCACUACUUCUUUUUCUGCCC CAGACGACUCGCCCGA	FILTER
8.15(2)	31	GGGAGGACGAUGCGG UUCCUCAAUUCUUCCAUCUUCAUAAUGUUUCCCUUUGCCC CAGACGACUCGCCCGA	FILTER
8.18(1)	32	GGGAGGACGAUGCGG UCUACCCUUUAGCAGUAUUUGUUUCCAUCGUUGUUUGCCC CAGACGACUCGCCCGG	NONE
8.20(1)	33	GGGAGGACGAUGCGG UCUCAACGAAGAACAUCGUUGGAUACUGUUUGUCCCGCCC CAGACGACUCGCCCGA	NONE
8.21(1)	34	GGGAGGACGAUGCGG UUCAGUUUCCUUCAGUUUUCGUUUCUAAUUCUUGUGUCCC CAGACGACUCGCCCGA	FILTER
8.22(1)	35	GGGAGGACGAUGCGG ----------AGCGGAUUAAUUAGUCUGACUUCUUGUCCC CAGACGACUCGCCCGA	
8.23(1)	36	GGGAGGACGAUGCGG AGACAUCUUUGUCUCGAUUAGUCAUGUUCCUUACCUGCCC CAGACGACUCGCCCGA	NONE
8.24(1)	37	GGGAGGACGAUGCGG --UCCUCUAGCAAGCAGCUUCUCAUCUUAUUUUUCCGCCC CAGACGACUCGCCCGA	
8.25(1)	38	GGGAGGACGAUGCGG UGCACAGUGAUGGAUGACAUUGUAUAACGGUAUGCGUCCC CAGACGACUCGCCCGA	
8.26(1)	39	GGGAGGACGAUGCGG -ACCUAUCUUUCUUCCAAGUCAUAGUUUUACUUCCCGCCC CAGACGACUCGCCCGA	FILTER
8.28(1)	40	GGGAGGACGAUGCGG AUGAGACCUAAUCAUCGAUCCGCUAUCUAAAACCUCACCC CAGACGACUCGCCCGA	NONE
8.29(1)	41	GGGAGGACGAUGCGG UCCUCAGACAAAUCUUUCUUGAAUCUUUCCUUAACUGCCC CAGACGACUCGCCCGA	FILTER
8.31(1)	42	GGGAGGACGAUGCGG -ACCGAUUCUCCAACUUGACAUUUAUUCCUCUUUCUGCCC CAGACGACUCGCCCGA	FILTER
8.33(1)	43	GGGAGGACGAUGCGG UCCUCUGAGCCAAUCUUCUUCGCUACUUCUUUUUCUGCCC CAGACGACUCGCCCGA	FILTER
8.34(1)	44	GGGAGGACGAUGCGG AUUCUUUCUCCAACGCUUUUCACUACCUACAUUUCUGCCC CAGACGACUCGCCCGA	FILTER
8.35(1)	45	GGGAGGACGAUGCGG AUCCUAUCCUCUGAAUAUCAUUAAAUCAUCUUCUCCGCCC CAGACGACUCGCCCGA	NONE

TABLE 5. (CONTINUED) Sequences isolated from round 8 of surface plasmon resonance SELEX.

8.36(1)	46	GGGAGGACGAUGCGG UUCAAUCAUCUUCACUCU-CAUUUCCUUUUUCCUACUCCC CAGACGACUCGCCCGA	FILTER
8.38(1)	47	GGGAGGACGAUGCGG CGAUAGAAUCUAGUCGUUCUAGAUGAUCUGGUACGUGCCC CAGACGACUCGCCCGA	
8.39(1)	48	GGGAGGACGAUGCGG UAGUAAUCCUUGUCUUCCAUUUCUCUUUACCCUUUUGCCC CAGACGACUCGCCCGA	FILTER
8.40(1)	49	GGGAGGACGAUGCGG ----CCCAUUAGUCCUCAUUAGU------CCCCUGUGCCC CAGACGACUCGCCCGA	NONE
8.41(1)	50	GGGAGGACGAUGCGG CAUCUUAUCCUCCAUCAGUUACUCUUCGUUAUUCCCGCCC CAGACGACUCGCCCGA	
8.45(1)	51	GGGAGGACGAUGCGG UCC-AAAUCCUCUUCCCAUGUUAGCAUUCAGCCUUGUCCC CAGACGACUCGCCCGA	
8.46(1)	52	GGGAGGACGAUGCGG -UUCCGACAAUUUCCUCCACCAUUAGAUUUCUUGCUGCCC CAGACGACUCGCCCGA	
8.47(1)	53	GGGAGGACGAUGCGG UCUUGAUCCUCCUUUGUGUCUUUCUUUGUCUUCCCUGCCC CAGACGACUCGCCCGA	
8.48(2)	54	GGGAGGACGAUGCGG AAGUAACGUUGA_AGUAAAAUUCGUUCUCUCGGU_AUU-_GGC CAGACGACUCGCCCGA	TGFβ2

NAME[a]	SEQ ID NO.	SEQUENCE[b]	BINDING[c]
8.49(1)	55	GGGAGGACGAUGCGG -UCCGAUCAGUUCCUUCGAUUAAUCUUCUUUCCUGCCCCC CAGACGACUCGCCCGA	
8.51(1)	56	GGGAGGACGAUGCGG AAUCCUUCUCCCCUGAUGAAUAUGACCUUUUUCUUGCUCCC CAGACGACUCGCCCGA	
8.52(1)	57	GGGAGGACGAUGCGG AUGAUCUUUAAUGUCUGGUUUGAGGUCAAUGCGGGUGCCC CAGACGACUCGCCCGA	
8.56(1)	58	GGGAGGACGAUGCGG AGAUGGUACUCCAUCUCCUUUAUGUGCCCAUCGCUGUCCC CAGACGACUCGCCCGA	
8.57(1)	59	GGGAGGACGAUGCGG UCCUC-GAUUCU---------AAUUUACUCCUUUUUCCCC CAGACGACUCGCCCGA	
8.61(1)	60	GGGAGGACGAUGCGG UCUACCCUUUAGCAGUAUUUGUUUCCAUCGUUGUUUGCCC CAGACGACUCGCCCGA	
8.62(1)	61	GGGAGGACGAUGCGG -CACAAUAUUCUCCUCUACUUCCACGUAUUUUCCUGUCCC CAGACGACUCGCCCGA	
8.64(1)	62	GGGAGGACGAUGCGG UCCUCAACCUUAGACUUUCAUUUCUUCAGUUCUUCUGCCC CAGACGACUCGCCCGA	
8.65(1)	63	GGGAGGACGAUGCGG UAGUGGUCUGUCAAAGGAAUAGCUAGUAGUGUUUGGUCCC CAGACGACUCGCCCGA	
8.69(1)	64	GGGAGGACGAUGCGG CAUCUUCCUUAGCAUACCAGUUUAUUCCUUUCCCUGUCCC CAGACGACUCGCCCGA	
8.71(1)	65	GGGAGGACGAUGCGG AGCGACAGUAUAGUUAGUACUCUAGCUCUAGUGCUGUCCC CAGACGACUCGCCCGA	
8.72(1)	66	GGGAGGACGAUGCGG ACCUCUCAUGAUCAGCAUCUCGCGUAAUCACGGUUCACCC CAGACGACUCGCCCGA	
8.74(1)	67	GGGAGGACGAUGCGG UCCGUACUCCAUUUCCUAUUUGAUUCCUUUUCCUCUGCCC CAGACGACUCGCCCGA	
8.75(1)	68	GGGAGGACGAUGCGG AACCCACGACCUUACCUUAAUCAUGUAUUUCUCUCUGCCC CAGACGACUCGCCCGA	

TABLE 5. (CONTINUED) Sequences isolated from round 8 of surface plasmon resonance SELEX.

8.76(1)	69	GGGAGGACGAUGCGG ------AGAUAAUGAGUGACGGUGAUUAUAGAUGCUGCCC CAGACGACUCGCCCGA
8.79(1)	70	GGGAGGACGAUGCGG UUCCUCAAUUCUUCCAUCUUCAUAAUGUUUCCCUUUGCCC CAGACGACUCGCCCGA
8.80(1)	71	GGGAGGACGAUGCGG UUCCU-------UCCAACGUUAUCUACUUUCU----GCCC CAGACGACUCGCCCGA

[a]Names are given in the form Round 8.clone number followed by the number of clones of that sequence that were isolated in parentheses.

[b]-, gaps introduced to designate sequences with selected regions that are shorter than 40 bases. An attempt was made to align such sequences with other sequences but the alignment is not necessarily optimal.

Underlined bases are those that differ from the ligand 14i-1 **(Table 7)**. A=2'-OH A; C=2'-F C; G=2'-OH G; U=2'-F U.

[C]FILTER, filter-binding sequence; NONE, no binding to TGFβ2 or filters, TGFβ2, binds to TGFβ2 as well as ligand 14i-1

TABLE 6. Conditions and results of resonant mirror (rm) optical biosensor SELEX.

Progress of IASYS SELEX with TGFβ2

Rd	TGFβ2, Arcsec[1]		[RNA], μM[2]	Vol, μL[3]	Binding (min)[4]	Dissociation (min)[5]	Elution[6]
	C1	C2					
10	1777	0	1	50	27	29	water
11	1777	0	10	50	30	60	water
12	1777	0	10	50	60	150	water
13	1893	0	0.05	50	37	73	water&SDS
14	1721	0	3.5	50	30	35	water&SDS

[1]Amount of TGFβ2 immobilized expressed in Arcsec where 1 Arcsec is 5 pg/mm^2 protein. The protein is immobilized in an area of 4 mm^2 in cell 1 (C1).

[2]Concentration of RNA pools

[3]Volume of RNA solution used

[4]Length of binding phase in min

[5]Length of dissociation phase in min

[6]Elution used

TABLE 7. Sequences isolated from round 13 of resonant mirror SELEX

NAME[a]	SEQ ID NO.	SEQUENCE [b]
14i-1	72	GGGAGGACGAUGCGG AAGUAACGUUGUAGUAAAAUUCGUUCUCUCGG-CAUUUGGC CAGACGACU-CGCCCGA
13.20(1)	73	GGGAGGACGAUGCGG AAGUAACGUUAUAGUAAAAUUCGUUCUCUCGG-UAUU_GGC CAGACGACU-CGCCCGA
13.22(2)	74	GGGAGGACGGUGCGG AAGUAACGUUGUAGUAAAAUUCGUUCUCUCGG-CGUUUGGC CAGACGACU-CGCCCGA
13.24(2)	75	GGGAGGACGAUGCGG AAGUAACGUUGUAGUAAAAUUCGUUCUCUCGG-CGUUUGGU CAGACGACU-CGCCCGA
13.30(1)	76	GGGAG_ACGAUGCGG AAGUAACGUUGUAGUAAAAUUCGUUCUCUCGG-CAUUUGGC CAGACGACU-CGCCCGA
13.32(1)	77	GGGAGGACGAUGCGG AAGUAACGUUGAAGUAAAAUUCGUUCUCUCUG-CGUUUGGU CAGACGACU-CGCCCGA
13.34(1)	78	GGGAGGACGAUGCGG AAGUAACGUUGAAGUAAAAUUCGUUCUCCUGG-UA_UUGGC CAGACGACU-CGCCCGA
13.36(2)	79	GGGAGGACGAUGCGG AAGUAACGUUGAAGUAAAAUUCGUUCUCUCGG-CAUUUGGC CAGACGACU-CGCCCGA
13.40(1)	80	GGGAGGACGAUGCGG AAGUAACGUUGUAGUAAAAUUCGUUCUCUUGG-CAUUU_GC CAGACGACU-CGCCCGA
13.42(1)	81	GGGAGGACGAUGCGG AAGUAACGUUAAAGUAAAAUUCGUUCUCUCGG-CGUUUGGC CAGACGACU-CGCCCGA
13.44(1)	82	GGGAGGACGAUGCGG AAGUAACGUUGAAGUAAAAUUCGUUCUCUCGG-CGUUUGGC CAGACGACU-CGCCCGA
13.48(1)	83	GGGAGGACGAUGCGG AAGUAACGUUGUAGUAAAAUUCGUUCUCUCGG-UAUUUGGC CAGACGACU-CGCCCGA
13.50(1)	84	GGGAGGACGAUGCGG AAGUAACGUUGUAGUAAAAUUCGUUCUCUUGG-UCUU_GGC CAGACGACU-CGCCCGA
13.54(1)	85	_GGAGGACGAUGCG_ AAGUAACGUUGUAGUAAAAUUCGUUCUCUCGGGCAUUUGG_ CAGACGACUUCGCCCGA

[a] Names are given in the form Round 13.clone number followed by the number of clones of that sequence that were isolated.

[b] Underlined bases are those that differ from ligand 14i-1 from the filter SELEX. The sequence of 14i-1 is shown at the top for comparison. A=2'-OH A; C=2'-F C; G=2'-OH G; U=2'-F U.

TABLE 8. Sequences and boundaries of TGFβ2 ligands isolated from rounds 14 and 21 of filter SELEX.

NAME [a]	SEQ ID NO.	SEQUENCE [b]	Kd (nM)	Ki (nM)
14i-1	72	**GGGAGGACGAUGCGGA**AGUAACGUUGUAGUAAAAUUCGUUCUCUCGGCAUUUGGC**CAGACGACUCGCCCGA**	10	230
21a-4	86	**GGGAGGACGAUGCGG**CGUUGUUUAGUCGUAUGUAUAUACUAAGUCCGCUUGUCCCC**AGACGACUCGCCCGA**	3	30
21a-21	87	**GGGAGGACGAUGCGG**-UUCAGGAGGUUAUUACAGAGUCUGUAUAGCUGUACUCCCC**AGACGACUCGCCCGA**	1	10
region:		5' fixed selected 3' fixed		

[a] Names are in the form: round sequence was isolated-clone number.

[b] Boundaries are underlined. Fixed regions are in bold-faced type. Selected sequences are in plain type.

A=2'-OH A; C=2'-F C; G=2'-OH G; U=2'-F U

TABLE 9. Number of sequences isolated using the SELEX process.

	SELEX round						
Sequence	8-spr	13-rm	14i	16a	18b	21a	TOTAL
14i-1	0	0	75	2	0	0	77
14i-1 variants	21	15	22	2	0	0	60
21a-4	0	0	0	0	0	3	3
21a-4 variants	0	0	4	7	0	2	13
21a-21	0	0	0	1	11	38	50
21a-21 variants	0	0	0	2	4	4	10
unidentified	36	0	0	0	0	0	36
filter-binding	12	0	1	1	0	1	15
TOTAL	69	15	102	15	15	48	264

TABLE 10. Characteristics of nucleic acid pools isolated using the SELEX method.

Round[a]	Sequence of pool[b]	% of pool[c]	% of transformants[d]	% of clones[e]
0	random	14i-1: <0.03		
6-spr	random	14i-1: ~1		
8-spr	slightly nonrandom	14i-1: ~5		14i-1: 30
				other: 70
9-spr	nonrandom			
9-rm	can read sequence of ligand 14i-1			
10-rm	can read sequence of ligand 14i-1			
11-rm	can read sequence of ligand 14i-1			
12-rm	can read variants of ligand 14i-1 sequence			
13-rm	can read variants of ligand 14i-1 sequence	14i-1: 10-100		14i-1: 100
		21a-21: <0.1		
14i				14i-1: 93
				21a-4: 4
		21a-21: 0.2-0.5		21a-21: 0
				other: 3
16a				14i-1: 27

TABLE 10. (CONTINUED) Characteristics of nucleic acid pools isolated using the SELEX method.

Round[a]	Sequence of pool[b]	% of pool[c]	% of transformants[d]	% of clones[e]
				21a-4: 47
		21a-21: 3-100		21a-21: 20
				other: 6
18b		21a-21: 3-100		21a-21: 100
21a				
			21a-4: 9	21a-4: 10
		21a-21: 3-100	21a-21: 90	21a-21: 84
			other: 1	other: 6

[a] spr, from surface plasmon resonance biosensor SELEX; rm, from resonant mirror optical biosensor SELEX.

[b] Determined by primer extension of bulk nucleic acid pools with 3'N7 primer.

[c] Determined by RT-PCR of bulk nucleic acid pools with a ligand-specific primer.

[d] Determined by PCR of individual transformants with a ligand-specific primer.

[e] Determined by sequencing of clones. Includes sequence variants of ligands.

TABLE 11. Truncates of human TGFβ2 nucleic acid ligand 21a-21.

NAME	SEQUENCE[a]	SEQ ID NO:	BINDING[b]	LENGTH[c]	BIO ACTIVITY[d]
21a-21	**GGGAGGACGAUGCGG**UUCAGG AGGUUAUUACAGAGUCUGUAUAGCUGUACUCCCC **AGACGACUCGCCCGA**	87	0.5	70	1
21a-21(U6G)	**GGGAGGACGAUGCGG**UUCAGGAGG**G** UAUUACAGAGUCUGUAUAGCUGUACUCCC**CAGACGACUCGCCCGA**	88	250	34	
21a-21Δ5'	***GG***UUCAGGAGGUUAUUACAGAGUCUGUAUAGCUGUACUCCC**CAGACGACUCGCCCGA**	89	0.5	56	
21a-21Δ3'	**GGGAGGACGAUGCGG**UUCAGGAGGUUAUUACAGAGUCUGUAUAGCUGUACUCCC**CA**	90	100	56	
21a-21Δ5',3'	***GG***UUCAGGAGGUUAUUACAGAGUCUGUAUAGCUGUACUCCC**CA**	91	0.5	42	1
21a-21(ML-94)	GGAGGUUAUUACAGAGUCUGUAUAGCUGUACUCCCC	92	0.5	36	
21a-21(ML-95)	GGAGGUUAUUACAGAGUCUGUAUAGCUGUACUCC	93	1	34	
21a-21(ML-96)	GGAGGUUAUUACAGAGUCUGUAUAGCUGUA	94	1000	30	
21a-21(ML-97)	GGAGGUUAUUACAGAGUCUGUAUAGC	95	1000	26	
21a-21(ML-99)	GGAGGUUAUUACAGAGUCUGUAUAGC CUCC	96	1000	30	
21a-21(ML-101)	GGAGGUUAUU AGAGUCU AUAGCUGUACUCC	97	1000	30	
21a-21(ML-102)	GGAGGUUAUU AGAGUCU AUAGC CUCC	98	1000	26	
21a-21(ML-103)	GGAGGUUAUUACAGAGUCUGUAUAGCUGUACUC	99	50	33	
21a-21(ML-104)	GGAGGUUAUUACAGAGUCUGUAUAGCUGUACU	100	70	32	
21a-21(ML-105)	GGAGGUUAUUACAGAGUCUGUAUAGCUGUAC	101	1000	31	
21a-21(ML-114)	GGAGGUUAUUACAGAGUCUGUAUAGC GUACUCC	102	1000	33	
21a-21(ML-115)	GGAGGUUAUUACAGAGUCUGUAUAGCUGU CUCC	103	1000	33	
21a-21(ML-116)	GGAGGUUAUUACAGAGUCUGUAUAGCU ACUCC	104	1000	32	
21a-21(ML-118)	GGAGGUUAU ACAGAGUCUGUAUAGCUGUACUCC	105	1000	33	
21a-21(ML-120)	GGAGGUUAUUACAGA UCUGUAUAGCUGUACUCC	106	1000	33	
21a-21(ML-122)	GGAGGUUAUUACA AGU UGUAUAGCUGUACUCC	107	1000	32	
21a-21(ML-128)	GGAGGUUAUUACAGAGU UGUAUAGCUGUACUCC	108	1000	33	

TABLE 11. (CONTINUED) Truncates of human TGFβ2 nucleic acid ligand 21a-21.

NAME	SEQUENCE[a]	SEQ ID NO:	BINDING[b]	LENGTH[c]	BIO ACTIVITY[d]
21a-21(ML-130)	GG GGUUAUUACAGAGUCUGUAUAGCUGUAC CC	109	2	32	
21a-21(ML-132)	GGAGGUUAUUAC GAGUCUGUAUAGC GUACUCC	110	1000	32	
21a-21(ML-134)	GGAGA UAUUACAGAGUCUGUAUAGCUGUACUCC	111	10	33	
21a-21(ML-136)	GG GGUUAUU CAGAGUCUGUAUAGCUG AC CC	112	10000	30	
21a-21(ML-138)	GG GGUUAUUA AGAGUCUGUAUAGCU UAC CC	113	10000	30	
NX22283	GGAGGUUAUUACAGAGUCUGUAUAGCUGUACUCCCC[3'T]	114	0.6	36	0.5
NX22284	GGAGGUUAUUACAGAGUCUGUAUAGCUGUACUCC [3'T]	115	1	34	1
NX22285	GGAGGUUAUUACAGAGUCUGUAUAGCUGUACUCCCCA	116	2	37	
NX22286	GGAGGUUAUUACAGAGUCUGUAUAGCUGUA	117	130	30	>20
NX22301	GAGGUUAUUACAGAGUCUGUAUAGCUGUACUCC [3'T]	118	1	33	2
NX22302	AGGUUAUUACAGAGUCUGUAUAGCUGUACUCC [3'T]	119	100	32	
NX22303	GGUUAUUACAGAGUCUGUAUAGCUGUACUCC [3'T]	120	>100	31	>100
NX22323	PEG-GGAGGUUAUUACAGAGUCUGUAUAGCUGUACUCC [3'T]	121	nt	34	3

[a] The fixed regions are indicated by bold-faced letters. The point mutant in 21a-21(U6G) is underlined and in bold type. A=2'-OH A; C=2'-F C; G=2'-OH G; U=2'-F U The italicized G at the 5' end of the 5' RNase H cleavage products indicates that ~50% of the time cleavage leaves 2 G's and 50% of the time one G is left. The boundaries in 21a-21 are underlined

[b] Binding is expressed as the ratio of the K_d of ligand /K_d of NX22284. The K_d of NX22284 is ~2 nM.

[c] Length is given in bases.

[d] Bioactivity is expressed as the ratio of the K_i of ligand /K_i of NX22284. The K_i of NX22284 is ~10 nM.

TABLE 12. Alignment of human transforming growth factor β amino acid sequences.

		SEQ ID NO.
TGFß1:	ALDTNYCFSS TEKNCCVRQL YIDFRKDLGW KWIHEPKGYH ANFCLGPCPY IWSLDTQYSK 60	122
TGFß2:	ALDAAYCFRN VQDNCCLRPL YIDFKRDLGW KWIHEPKGYN ANFCAGACPY LWSSDTQHSR 60	123
TGFß3:	ALDTNYCFRN LEENCCVRPL YIDFRQDLGW KWVHEPKGYY ANFCSGPCPY LRSADTTHST 60	124
TGFß2 specific:	AA VQD L KR N A A S R	
TGFß1:	VLALYNQHNP GASAAPCCVP QALEPLPIVY YVGRKPKVEQ LSNMIVRSCK CS 112	125
TGFß2:	VLSLYNTINP EASASPCCVS QDLEPLTILY YIGKTPKIEQ LSNMIVKSCK CS 112	126
TGFß3:	VLGLYNTLNP EASASPCCVP QDLEPLTILY YVGRTPKVEQ LSNMVVKSCK CS 112	127
TGFß2 specific:	S I S I K I	

TABLE 13. Truncates of human TGFβ2 nucleic acid ligand 14i-1.

NAME	SEQUENCE[a]	SEQ ID NO.	BINDING[b]	LENGTH[c]
14i-1	**GGGAGGACGAUGCGG**AAGUAACGUUGUAGUAAAAUUCGUUCUCUC GGCAUUUGGC**CAGACGACUCGCCCGA**	72	1	71
14i-1Δ5'[d]	***GG***AAGUAACGUUGUAGUAAAAUUCGUUCUCUCGGCAUUUGGC**CAGACGACUCGCCCGA**	128	>100	56
14i-1Δ3'[d]	**GGGAGGACGAUGCGG**AAGUAACGUUGUAGUAAAAUUCGUUCUCUCGGCAUUUGGC**CA**	129	3	57
14i-1Δ5,3'[d]	***GG***AAGUAACGUUGUAGUAAAAUUCGUUCUCUCGGCAUUUGGC**CA**	130	>100	42
14i-1t5-41	gGGAgGAUGCGGAAGUAACGUUGUAGUAAAAUUCcUUC	131	1	38
14i-1t5-38	gGGAgGAUGCGGAAGUAACGUUGUAGUAAAAUUCc	132	>100	35
14i-1t5-35	gGGAgGAUGCGGAAGUAACGUUGUAGUAAAAU	133	>100	32
14i-1(ML-86)	gGGAgGAUGCGGAAGUAACGUUGUAGU UCcUUC	134	>100	33
14i-1(ML-87)	gGGAgGAUGCGGAAGUAACGUUGUAGU	135	>100	27
14i-1(ML-89)	gGgaGgAGUAACGUUGUAGU	136	>100	20

[a] Lowercase letters indicate bases not found at that position in the full length ligand that were added or changed to maintain transcriptional efficiency. Boundaries are underlined. The fixed regions are in bold-faced type. The italicized G at the 5' end of the 5' RNase H cleavage products indicates that ~50% of the time cleavage leaves 2 G's and 50% of the time one G is left. A=2'-OH A; C=2'-F C; G=2'-OH G; U=2'-F .

[b] Binding is expressed as the ratio of K_d (ligand)/Kd (14i-1). The K_d of 14i-1 is ~10 nM.

[c] Length is in bases.

[d] Produced by RNase H digestion.

TABLE 14. Truncates of human TGFβ2 nucleic acid ligand 21a-4.

Name	Sequence[a]	SEQ ID NO.	Binding[b]	Length[c]
21a-4	**GGGAGGACGAU**<u>**G**</u>**CGG**CGUUGUUUAGUCGUAUGUAUAUACUAAGUCCGCUUGU<u>CCCC</u>**AGACGACUCGCCCGA**	86	1	71
21a-4Δ5'[d]	***GG***CGUUGUUUAGUCGUAUGUAUAUACUAAGUCCGCUUGUCCC**CAGACGACUCGCCCGA**	137	>100	56
21a-4Δ3'[d]	**GGGAGGACGAUGCGG**CGUUGUUUAGUCGUAUGUAUAUACUAAGUCCGCUUGUCCC**CA**	138	1	57
21a-4Δ5',3'[d]	***GG***CGUUGUUUAGUCGUAUGUAUAUACUAAGUCCGCUUGUCCC**CA**	139	>100	42
21a-4(ML-91)	gg**G**ga**GCGG**CGUUGUUUAGUCGUAUGUAUAUACUAAGUCCGCUU	140	1	44
21a-4(ML-92)	gg**G**ga**GCGG**CGUUGUU gaaa AGUCCGCUU	141	>100	27
21a-4(ML-108)	gg**G**ga**GCGG**CGUUGUUU CGUAUGUAUAU AAGUCCGCUU	142	>100	38
21a-4(ML-109)	gg**G**ga**GCGG**CGUUGUUU AUGUAU AAGUCCGCUU	143	>100	33
21a-4(ML-110)	gg**G**ga**GCGG**CGUUGUUUAGUCGUAUGUAUAUACUAAGUCCGC	144	1	42
21a-4(ML-111)	gg**G**ga**GCGG**CGUUGUUUAGUCGUAUGUAUAUACUAAGU	145	30	38

a Lowercase letters indicate bases not found at that position in the full length ligand. Underlining indicates boundary positions. The fixed region sequences are indicated in bold-faced lettering. The italicized G at the 5' end of the 5' RNase H cleavage products indicates that ~50% of the time cleavage leaves 2 Gs and 50% of the time one G is left. A=2'-OH A; C=2'-F C; G=2'-OH G; U=2-F U

b Binding is expressed as the ratio of K_d (ligand)/K_d (21a-4). The K_d of 21a-4 is ~3 nM.

c Length is expressed in bases.

d These ligands were generated by RNAse H digestion of 21a-4.

TABLE 15. Biased SELEX conditions and results.

Round[a]	[RNA][b], nM	[TGFβ2], nM	RNA[b]/protein	[Competitor]	% Bound	% Background	Bound/background	Kd (nM)[c]
34N7.21a-21 round 0 nucleic acid								870
1a	1000	150	7	0	1.4	1.4	1.0	395
2a	450	300	1.5	0	1.7	1.0	1.7	186
3a	10	50	0.2	0	17.5	1.0	17.5	25
4a	50	10	5	0	11.0	0.9	12.3	17
4b	50	10	5	333 nM NX22284	2.2	1.3	1.7	8
5a	8	1	8	0	1.4	0.9	1.5	1
5b	8	1	8	100 nM NX22284	0.8	0.7	1.1	17
6a	4	0.5	8	0	2.9	2.9	1.0	1
6b	6	0.5	12	100 nM NX22284	1.8	1.3	1.4	1
7a	5	0.25	20	0	0.5	0.14	3.4	1
7b	5	0.25	20	200 nM NX22284 5 mM tRNA	0.15	0.1	1.5	0.5
8a	1	0.05	20	0	1.05	1.1	0.9	1
8b	1	0.05	20	100 nM NX22284 5 mM tRNA	0.6	0.5	1.2	3
9a	125	1	125	0	0.6	0.5	1.2	nd
9b	0.9	0.01	90	0	0.15	0.14	1.0	nd

[a] a series, without competitor; b series, with competitors

[b] nucleic acid ligand library

[c] nd, not determined

TABLE 16. Nucleic acid ligands isolated from round 5a of a human TGFβ2 biased SELEX.

NAME[a]	5′ FIXED	SELECTED[b]	3′ FIXED	SEQ ID NO:	CHANGES[c]	BINDING[d]
putative structural element:		S1 B S2 L S2 S1				
21a-21:	GGGAGGACGAUGCGGUUCA	GGAG GUUAUUACAGAGUCUGUAUAG CUGUACUCCC	CAGACGACUCGCCCGA	72	0	1.0
1: (2)	GGGAGGACGAUGCGG	GG**U**GA**U**UUAUUACAGAGU**A**UGUAUAGCUGUAC**C**CC	CAGACGACUCGCCCGA	146	4	0.8
2: (1)	GGGAGGACGAUGCGG	**AGGC**GUUAUUA**G**AGAGUCUGUAUAGCU**C**UA**GC**CC	CAGACGACUCGCC-GA	147	7	0.6
4: (1)	GGGAGGACGAUGCGG	GGAGG**G**UAUUACAGAGU**A**UGUAUAGCUGUACUCC	CAGACGACUCGCCCGA	148	2	1.4
6 :(2)	GGGAGGACGAUGCGG	GGAGGUUAUUA**U**AGAGUCUGUAUAGCU**A**UACCCC	CAGACGACUCGCCCGA	149	3	1.6
7: (1)	GGGAGGACGAUGCGG	G**AG**GGUUAUUA**U**AGAGUCUG**C**AUAGCU**A**UACCCC	CAGACGACUCGCCCGA	150	5	0.3
9: (1)	GGGAGGACGAUGCGG	**U**GAG**AG**UAUUAC**G**GAGU**A**UGUAUAGC**C**GUACCCC	CAGACGACUCGCCCGA	151	7	0.3
10:(1)	GGGAGGACGAUGCGG	GG**GCA**UUAUUU**C**AGAGUCUGUAUAGCUGUA**GC**CC	CAGACGACUCGCCCGA	152	6	0.3
11:(2)	GGGAGGACGAUGCGG	G**CGGA**UUAU**C**ACAGAGU**A**UGUAUAGCUGU**GCCG**C	CAGACGACUCGCCCGA	153	8	0.4
13:(1)	GGGAGGACGAUGCGG	**UGUGAA**UAUUA**G**AGAGUCUGUAUAGCU**C**UACCCC	CAGACGACUCGCCCGA	154	7	0.2
14:(1)	GGGAGGACGAUGCGG	**CGGGA**UUAUUAC**U**GAGUCUGUAUAGC**A**GUACCCC	CAGACGACUCGCCCGA	155	6	0.4
15:(1)	GGGAGGACGAUGCGG	G**UGGAA**UAUUAC**G**GAGUCUGUAUAGC**C**GUACUCC	CAGACGACUCGCCCGA	156	6	0.4
17:(1)	GGGAGGACGAUGCGG	GGGG**AC**UAUUA**GU**GAGUCUGUAUAGC**AC**UACCCC	CAGACGACUCGCCCGA	157	8	0.8
18:(1)	GGGAGGACGAUGCGG	G**UGGA**UUAUUACAGC**G**UCUGUAUA**U**CUGUACCCC	CAGACGACUCGCCCGA	158	6	1.0
19:(2)	GGGAGGACGAUGCGG	GC**A**GGUUAUUACAGAGUCUGUAUAGCUGUACU**GC**	CAGACGACUCGCCCGA	159	2	1.0
20:(1)	GGGAGGACGAUGCGG	GG**UAGA**UAU**C**AC**U**GAGUCUGUAUAGC**A**GU**GUC**CC	CAGACGACUCGCCCGA	160	9	5.7
21:(2)	GGGAGGACGAUGCGG	**AGGGA**UUAUUACAGAGUCUGUAUAGCUGUAC**C**CC	CAGACGACUCGCCCGA	161	4	0.7
22:(4)	GGGAGGACGAUGCGG	G**UGGA**UUAUUACAGAGUCUGUAUAGCUGUAC**C**CC	CAGACGACUCGCCCGA	162	4	1.1
25:(1)	GGGAGGACGAUGCGG	GG**GC**GUUAUUACAGAGUCUGUAUAGCUGUA**GC**CC	CAGACGACUCGCCCGA	163	4	1.0
26:(1)	GGGAGGACGAUGCGG	GG**U**GGUUAUUACA**C**AGU**A**UGUAUAG**G**UGUAC**C**CC	CAGACGACUCGCCCGA	164	4	3.1
28:(1)	GGGAGGACGAUGCGG	**AGGGAA**UAUUACAGAGU**A**UGUAUAGCUGUAC**C**CC	CAGACGACUCGCCCGA	165	6	1.0
29:(1)	GGGAGGACGAUGCGG	GGAG**U**UUAUUACAGC**G**UCUGUAUA**U**CUGUA**GC**CC	CAGACGACUCGCCCGA	166	5	1.0
30:(1)	GGGAGGACGAUGCGG	**U**GAGGUUAUUACAGAGUCUGUAUAGCUGUACUCC	CAGACGACUCGCCCGA	167	1	2.4
34:(1)	GGGAGGACGAUGCGG	GG**U**GGUUAUUA**G**AGAGUCUGUAUAGCU**C**UAC**G**CC	CAGACGACUCGCCCGA	168	4	1.7

TABLE 16 CONT.

35:(1)	GGGAGGACGAUGCGG	GG**GG**A**G**UAUUA**AA**GAGUCUGUAUAGCU**U**UAC**C**CC	CAGACGACUCGCCCGA	169	6	0.8
36:(1)	GGGAGGACGAUGCGG	GGAGG**A**UAUUA**U**AGAGUCUGUAUAGCU**A**UAC**C**CC	CAGACGACUCGCCCGA	170	4	1.9
invariant:		UAU GU UG AUA C				

[a] Number of clones isolated for each sequence is indicated in parentheses.

[b] Nucleotides that differ from the starting sequence are shown in bold-faced lettering. A=2'-OH A; C=2'-F C; G=2'-OH G; U=2'-F U

Putative structural elements: S1, stem 1; B, bulge; S2, stem 2; L, loop. The sequence of ligand 21a-21 is shown at the top for comparison.

[c] Number of changes from starting sequence.

[d] Binding is expressed as K_d (ligand)/ K_d (21a-21). The K_d of ligand 21a-21 is about 1 nM.

TABLE 17. Highest and lowest affinity TGFβ2 nucleic acid ligands from biased SELEX.

NAME	5′ FIXED	SELECTED[a]	3′ FIXED	SEQ ID NO.	BINDING[b]	CHANGES[c]
HIGHEST AFFINITY LIGANDS:						
13:	GGGAGGACGAUGCGG	**U**G**U**G**AA**UAUUA**G**AGAGUCUGUAUAGCU**C**UAC**C**CC	CAGACGACUCGCCCGA	154	0.2	7
14:	GGGAGGACGAUGCGG	**C**GG**G**AUUAUUAC**U**GAGUCUGUAUAGC**A**GUAC**C**CC	CAGACGACUCGCCCGA	155	0.4	6
21:	GGGAGGACGAUGCGG	**A**GG**G**AUUAUUACAGAGUCUGUAUAGCUGUAC**C**CC	CAGACGACUCGCCCGA	161	0.7	4
35:	GGGAGGACGAUGCGG	GG**G**G**AG**UAUUA**A**AGAGUCUGUAUAGCU**U**UAC**C**CC	CAGACGACUCGCCCGA	169	0.8	6
putative structural elements:		S1 B S2 L S2 S1				
21a-21:	GGGAGGACGAUGCGGUUCAGGAG	GUUAUUACAGAGUCUGUAUAG	CUGUACUCCC	CAGACGACUCGCCCGA	72	1.0	0
LOWEST AFFINITY LIGANDS:						
36:	GGGAGGACGAUGCGG	GGAGG**A**UAUUA**U**AGAGUCUGUAUAGCU**A**UAC**C**CC	CAGACGACUCGCCCGA	170	2.0	4
30:	GGGAGGACGAUGCGG	**U**GAGGUUAUUACAGAGUCUGUAUAGCUGUACUCC	CAGACGACUCGCCCGA	167	2.4	1
26:	GGGAGGACGAUGCGG	GG**U**GGUUAUUACA**C**AGU**A**UGUAUAGG**G**UGUAC**C**CC	CAGACGACUCGCCCGA	164	3.1	4
6:	GGGAGGACGAUGCGG	GGAGGUUAUUA**U**AGAGUCUGUAUAGCU**A**UAC**C**CC	CAGACGACUCGCCCGA	149	3.3	3
20:	GGGAGGACGAUGCGG	GG**U**AGAUAU**C**AC**U**GAGUCUGUAUAGC**A**GU**GU**CCC	CAGACGACUCGCCCGA	160	5.7	9
invariant:		UAU GU UG AUA C				

[a] Nucleotides that differ from the starting sequence are shown in bold-faced lettering. A=2'-OH A; C=2'-F C; G=2'-OH G; U=2'-F U
Putative structural elements: S1, stem1; B, bulge; S2, stem2; L, loop.

[b] Binding is expressed as K_d (ligand)/ K_d (21a-21). The K_d of 21a-21 is 1 nM

[c] Number of changes from starting sequence.

TABLE 18. Substitution of 2'-OH purines with 2'-OCH_3 purines in NX22284 ligand.

NAME	SEQUENCE[a]	SEQ ID NO.	BINDING[b]	LENGTH[c]	BIOACTIVITY[d]
NX22284	GGAGGUUAUUACAGAGUCUGUAUAGCUGUACUCC[3'T]	115	1	34	1
NX22304	ggaggUUaUUaCagagUCUgUaUagCUgUaCUCC[3'T]	171	>100	34	>100
NX22355	GGAGGUUAUUaCagagUCUgUaUagCUgUaCUCC[3'T]	172	>100	34	>100
NX22356	ggagGUUAUUACAGAGUCUGUAUAGCUGUACUCC[3'T]	173	1	34	1
NX22357	GGAGgUUaUUaCAGAGUCUGUAUAGCUGUACUCC[3'T]	174	2	34	10
NX22358	GGAGGUUAUUACagagUCUGUAUAGCUGUACUCC[3'T]	175	1	34	1
NX22359	GGAGGUUAUUACAGAGUCUgUaUaGCUGUACUCC[3'T]	176	>100	34	>30
NX22360	GGAGGUUAUUACAGAGUCUGUAUAgCUgUaCUCC[3'T]	177	1	34	1
NX22374	GGAGGUUAUUACAGAGUCUgUAUAGCUGUACUCC[3'T]	178	25	34	>100
NX22375	GGAGGUUAUUACAGAGUCUGUaUAGCUGUACUCC[3'T]	179	>100	34	>300
NX22376	GGAGGUUAUUACAGAGUCUGUAUaGCUGUACUCC[3'T]	180	50	34	>100
NX22377	ggaggUUaUUaCAGAGUCUGUAUAgCUgUaCUCC[3'T]	181	1	34	1
NX22383	ggaggUUaUUaCagagUCUGUAUagCUgUaCUCC[3'T]	182	500	34	>100
NX22384	ggaggUUaUUaCagagUCUgUAUagCUgUaCUCC[3'T]	183	10000	34	>100
NX22417	ggaggUUaUUaCagagUCUGUAUAgCUgUaCUCC[3'T]	184	1	34	10
NX22420	ggaggUUAUUaCagagUCUGUAUAgCUgUaCUCC[3'T]	185	1	34	1
NX22421	ggagGUUAUUACagagUCUGUAUAgCUgUaCUCC[3'T]	186	2	34	1
NX22426	ggaga-UAUUaCagagUCUGUAUAgCUgUaCUCC[3'T]	187	1	33	25
NX22427	gg-ggUUAUUaCagagUCUGUAUAgCUgUaC-CC[3'T]	188	0.3	32	0.7

TABLE 18 CONT.

[a] A, 2'-OH A; C, 2'-F C; G, 2'-OH G; U, 2'-F U; a, 2'-OCH_3 A; g, 2'-OCH_3 G. [3'T] signifies a 3', 3' dT cap.

[b] Binding is expressed as the ratio of the K_d of ligand /K_d of NX22284. The K_d of NX22284 is ~1 nM.

[c] Length is given in bases.

[b] Bioactivity is expressed as the ratio of the K_i of ligand /K_i of NX22284. The K_i of NX22284 is ~10 nM.

TABLE 19. Truncates and 2'-OCH_3 purine modifications of nucleic acid ligand #13 from a biased SELEX.

NAME	SEQUENCE[a]	SEQ ID NO.	BINDING[b]		LENGTH[c]
BIOACTIVITY[d]					
NX22385	UGUGAAUAUUAGAGAGUCUGUAUAGCUCUACCCC[3'T]	189	0.4	34	4
NX22386	UgUgaAUaUUaGagagUCUGUAUagCUCUaCCCC[3'T]	190	3000	34	>100
NX22387	UgUgaaUaUUagagagUCUgUAUagCUCUaCCCC[3'T]	191	3000	34	30
NX22424	UgUgAAUAUUaGagagUCUGUAUAgCUCUaCCCC[3'T]	192	0.6	34	>100
NX22425	UgUgaaUAUUagagagUCUGUAUAgCUCUaCCCC[3'T]	193	1.5	34	>100

[a] A, 2'-OH A; C, 2'-F C; G, 2'-OH G; U, 2'-F U; a, 2'-OCH_3 A; g, 2'-OCH_3 G. [3'T] signifies a 3', 3' dT cap.

[b] Binding is expressed as the ratio of the K_d of ligand/K_d of NX22284. The K_d of NX22284 is 2 nM.

[c] Length is given in bases.

[b] Bioactivity is expressed as the ratio of the K_i of ligand/K_i of NX22284. The K_i of NX22284 is 10 nM.

TABLE 20. Pharmacokinetic properties of NX22323 in rats using a noncompartmental analysis.

Parameter	Units	Estimate
Cmax	(μg/mL)	27.1
AUClast	((μg*min)/mL)	3028.0
AUCINF	((μg*min)/mL)	3058.0
Beta t1/2	(min)	630.9
Cl	(mL/(min*kg))	0.33
MRTINF	(min)	350.4
Vss	(mL/kg)	115.0
Vz	(mL/kg)	298.0

TABLE 21. Pharmacokinetic properties of NX22323 in rats using a compartmental analysis.

Parameter	Units	Estimate	StdError	% Error
Cmax	(μg/mL)	16.3	3.3	20.2
AUCINF	((μg*min)/mL)	2486	274	11.0
Alpha-t1/2	(min)	63.5	19.1	30.2
Beta-t1/2	(min)	467.2	83.2	17.8
A	(μg/mL)	14.63	3.21	21.9
B	(μg/mL)	1.70	0.84	49.1
Cl	(mL/(min*kg)	0.402	0.044	11.0
MRTINF	(min)	360.3	35.6	9.9
Vss	(mL/kg)	144.9	23.1	15.9

TABLE 22. Binding and inhibitory activity of 2'-Omethyl- and Pegyl-modifications of lead TGFβ1 truncate ligand CD70

		SEQ ID NO.	Binding	Bioactivity
ChD70	GGGUGCCUUUUGCCUAGGUUGUGAUUUGUAACCUUCUGCCCA	216	+++	+++
ChD70-m1	**ggg**U**g**CCUUUUGCCUAGGUUGUGAUUUGUAACCUUCUGCCCA	194	+	
ChD70-m2	GGGUGCCUUUU**g**CCU**agg**UUGUGAUUUGUAACCUUCUGCCCA	195	++	
ChD70-m3	GGGUGCCUUUUGCCUAGGUU**g**U**ga**UUU**g**UAACCUUCUGCCCA	196	+++	
ChD70-m4	GGGUGCCUUUUGCCUAGGUUGUGAUUUGU**aa**CCUUCU**g**CCC**a**	197	++	
ChD70-m5	**g**GGUGCCUUUUGCCUAGGUU**g**U**ga**UUU**g**UAACCUUCUGCCCA	198	+++	
ChD70-m6	G**g**GUGCCUUUUGCCUAGGUU**g**U**ga**UUU**g**UAACCUUCUGCCCA	199	+++	
ChD70-m7	GG**g**UGCCUUUUGCCUAGGUU**g**U**ga**UUU**g**UAACCUUCUGCCCA	200	+++	
ChD70-m8	GGGU**g**CCUUUUGCCUAGGUU**g**U**ga**UUU**g**UAACCUUCUGCCCA	201	+	
ChD70-m9	GGGUGCCUUUU**g**CCUAGGUU**g**U**ga**UUU**g**UAACCUUCUGCCCA	202	+	
ChD70-m10	GGGUGCCUUUUGCCU**a**GGUU**g**U**ga**UUU**g**UAACCUUCUGCCCA	203	+++	
ChD70-m11	GGGUGCCUUUUGCCUA**g**UU**g**U**ga**UUU**g**UAACCUUCUGCCCA	204	+++	
ChD70-m12	GGGUGCCUUUUGCCUAG**g**UU**g**U**ga**UUU**g**UAACCUUCUGCCCA	205	+++	
ChD70-m13	GGGUGCCUUUUGCCUAGGUU**g**U**ga**UUU**g**U**a**ACCUUCUGCCCA	206	+++	
ChD70-m14	GGGUGCCUUUUGCCUAGGUU**g**U**ga**UUU**g**UA**a**CCUUCUGCCCA	207	+++	
ChD70-m15	GGGUGCCUUUUGCCUAGGUU**g**U**ga**UUU**g**UAACCUUCU**g**CCCA	208	+++	
ChD70-m16	GGGUGCCUUUUGCCUAGGUU**g**U**ga**UUU**g**UAACCUUCUGCCC**a**	209	+++	
ChD70-m17	**ggg**UGCCUUUUGCCU**agg**UU**g**U**ga**UUU**g**U**aa**CCUUCUGCCC**a**3'-3'U	210	+++	+++
ChD70-m18	**ggg**UGCCUUUUGCCU**agg**UU**g**U**ga**UUU**g**U**a**ACCUUCUGCCC**a**3'-3'U	211	+++	
ChD70-m19	**ggg**UGCCUUUUGCCU**agg**UU**g**U**ga**UUU**g**U**aa**CCUUCUGCCC3'-3'U	212	++	-

TABLE 22 CONT. Binding and inhibitory activity of 2'-Omethyl- and Pegyl-modifications of lead TGFβ1 truncate ligand CD70

ChD70-m20	**ggg**UGCCUUUUGCCU**agg**UU**g**U-----**g**U**aa**CCUUCUGCCC**a**3'-3'U	213	++
ChD70-m21	**ggg**UGCCUUUUGCCU**agg**UU**g**-------U**aa**CCUUCUGCCC**a**3'-3'U	214	++
ChD70-m22	**ggg**UGCCUUUUGCCU**agg**UU---------**aa**CCUUCUGCCC**a**3'-3'U	215	+++

Lower case-bold residues indicate 2'Omethyl substitutions. The gap shown was occupied by a PEG linker (spacer 18 Glen Research). Number of (+) indicate extent of binding or inhibition of TGFβ1 bioactivity.